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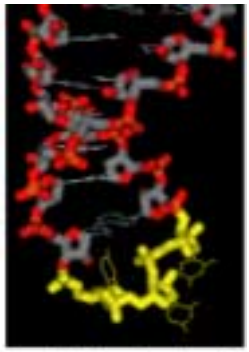
nature REVIEWS Story contributed by Tanita Casci, Nature Reviews Genetics



The anticodon stem-loop of tRNA^{lys}. Three bases on each tRNA provide the foundation for the genetic code.

Click on the figure for more information.

Cytosolic help for mitochondrial defects

The mitochondrion has cut back its genome substantially since taking up residence in cells as a symbiont 1.5 billion years ago, but it retains its personal transcription, translation and protein-assembling systems, including its tRNA genes. Even so, the mitochondrion is not fully self-sufficient to varying extents yeast, plants and protozoan cells can borrow nuclear-encoded tRNA molecules to ease the task of translating transcripts of their mitochondrial genes. New data indicate that nuclear-encoded tRNAs can even be used to salvage errors in mitochondrial transcripts.

In the yeast *Saccharomyces cerevisiae,* only one tRNA ($tRNA^{Lys}_{CUU}$) is carried into the mitochondrion, something it can do only if charged with an amino acid, and only if aided by cytosolic import factors. Among these factors is the precursor of the mitochondrial lysyl-tRNA synthetase (pre-MSK).

In a recent publication, researchers altered the aminoacylation identity of $tRNA^{Lys}_{CUU}$ so that it was charged with methionine rather than lysine. Both in live yeast cells and in isolated mitochondria, the engineered tRNA could enter the mitochondrion, where the radiolabelled methionine charged on the imported tRNA was incorporated normally into mitochondrial proteins. A second, modified $tRNA^{Lys}$ version with alanine identity was also successfully used *in vivo* to suppress an *amber* (UAG) stop codon (a nonsense mutation) in the mitochondrial *COX2* gene.

Defects in mitochondrial (mt) DNA, caused by base substitutions or rearrangements in genes that encode proteins or tRNAs underlie a range of human pathologies (as discussed in the previous highlight).

Could the technique used to modify mitochondrial mutations be adapted for use in humans, given that import of nuclear-encoded tRNAs into mammalian mitochondria has never been seen? It seems so, because isolated human mitochondria imported the yeast $tRNA^{Lys}_{CUU}$ and its derivatives, provided that the human cytosolic extracts were supplemented with the yeast pre-MSK. The foreign tRNA was functional on the translational apparatus of human mitochondria, just as in yeast.

This recent innovation might be useful for replacing non-functional tRNAs or for suppressing nonsense mutations in mtDNA.

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Cytosolic help for mitochondrial defects

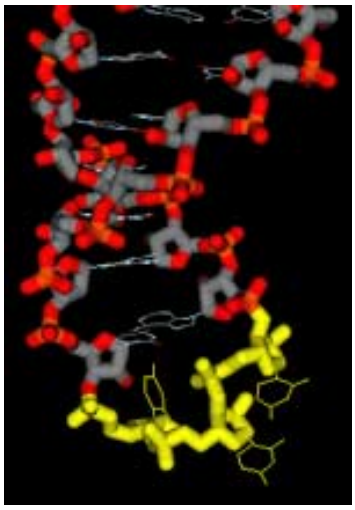

Figure 1. Tertiary structure of tRNA^{Lys}. The anticodon loop and stem of tRNA^{Lys} is depicted in the image to the left. The three bases that compose the anticodon (in this example, "U-U-U") are highlighted in yellow.

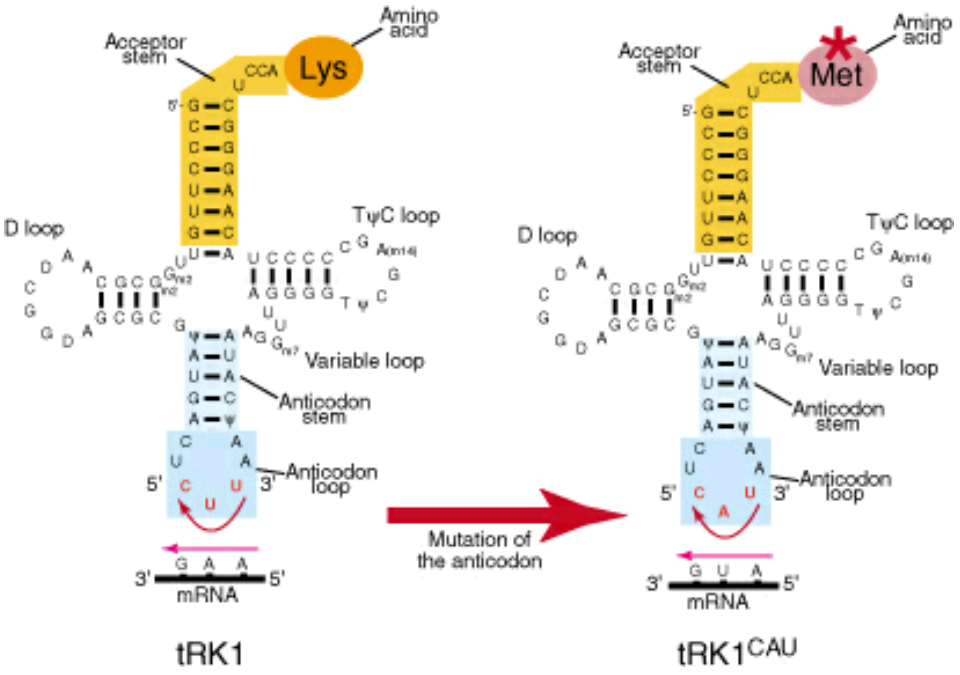

Figure 2. Mutation of the anticodon of tRNA^{Lys} from C-U-U to C-A-U. Outside of the mitochondria, tRNA$^{Lys}_{CUU}$ is charged with lysine, its cognate amino acid. However, by changing the anticodon from C-U-U to C-A-U, the mutated tRNA is subsequently charged with methionine. To be able to track mutant tRNAs, an ^{35}S-radiolabelled methionine was used. This experiment demonstrated that aminoacylation was necessary for transport of tRNA into the mitochondria, although the identity of the amino acid that was charged onto the tRNA was less important.

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This is the Organelle Genome Resources page. To obtain information on Metazoa mitochondrial genomes, click on the link marked by the red arrow to the left. (Note: selecting other links will take you out of this tutorial.)

Mitochondria and **chloroplasts** (a type of plastid) are membrane-bound organelles that convert energy from foodstuffs (mitochondria) or sunlight (chloroplasts) into forms that can be used by the cell. The organelles likely evolved from bacteria that were endocytosed more than one billion years ago. Although the organelles maintain their own genomes, many genes encoding mitochondrial and chloroplast proteins are found in the cell nucleus.

Mitochondria are small, oval shaped organelles surrounded by two highly specialized membranes. Mitochondria are the sites of aerobic respiration, and are generally the major energy production center in eukaryotes. Animal mitochondrial genomes are normally circular, ~16 kB in length, and encode 13 proteins used for energy production, as well as 22 tRNAs and 2 rRNAs. Plant mitochondrial genomes tend to be 10 - 150 times larger and contain additional genes. Many organisms use one genetic code to translate nuclear mRNAs, and a second one for their mitochondrial mRNAs.

Chloroplasts are larger than mitochondria, and are surrounded by three specialized membranes. In plants and some other eukaryotes, chloroplasts are the sites of photosynthesis, a process in which atmospheric carbon dioxide is "fixed" into organic compounds, and oxygen is released into the atmosphere. Chloroplast genomes are ~120 - 200 kB in length. Their ~120 genes encode ribosomal RNAs and proteins, tRNAs, and proteins involved in photosynthesis. Chloroplast mRNAs are translated with the standard genetic code, although they often undergo extensive RNA editing, so it is difficult to predict the protein translations from genomic sequence.

The organelle genomes on this site are part of the NCBI Reference Sequence (RefSeq) project that provides curated sequence data and related information for the community to use as a standard. The animal (metazoan) mitochondrial records are considered "reviewed", that is, they have been manually curated by the NCBI staff. Other mitochondrial and chloroplast genome records are "provisional" and are presented as found in the source GenBank records used to create them.

The mitochondion and chloroplast images are courtesy of The Biology Project.

Metazoa that have their complete mitochondrial genomes sequenced are listed below. The taxonomic names provide links to the NCBI Taxonomy database. The numbers in green show the number of organisms at each node with sequenced mitochondrial genomes.

To continue with the tutorial, click on the "[40]" indicated by the red arrow to the right of the *Mammalia* node.

Metazoa mitochondrial genomes

- ***Metazoa*** [121]
  - ***Bilateria*** [120]
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      - ***Platyhelminthes*** [7] (flatworms)
    - ***Coelomata*** [110]
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            - ***Actinopterygii*** [12] (ray-finned fishes)
            - ***Chondrichthyes*** [4](cartilaginous fishes)
            - ***Coelacanthiformes*** [1](coelacanths)
            - ***Dipnoi*** [1](lungfishes)
            - ***Hyperoartia*** [2](lampreys)
            - ***Tetrapoda*** [58]
              - ***Amphibia*** [2](amphibians)
              - ***Mammalia*** [40] (mammals)
              - ***Sauropsida*** [16](sauropsids)
        - ***Echinodermata*** [5](echinoderms)
        - ***Hemichordata*** [1](hemichordates)
      - ***Protostomia*** [23]
        - ***Annelida*** [2](annelid worms)
        - ***Arthropoda*** [13]
        - ***Brachiopoda*** [2](lampshells)
        - ***Mollusca*** [6]
    - ***Pseudocoelomata*** [3]
      - ***Nematoda*** [3](roundworms)
  - ***Cnidaria*** [1]

Members of the class *Mammalia* with sequenced mitochondrial genomes are listed below. Each organism is linked to the NCBI Taxonomy database and Entrez genome. To view the mitochondrial gene order of these species, click on "Gene/RNA order" located on the navigation bar to the left.

Mammalia mitochondrial genomes - 40 records

Tetrapoda

Organism	Accession	Length	Protein	RNA	Date	
Artibeus jamaicensis	NC_002009	16651 bp	13	24	8/24/1999	(Jamaican fruit-eating bat)
Balaenoptera musculus	NC_001601	16402 bp	13	24	8/24/1999	(blue whale)
Balaenoptera physalus	NC_001321	16398 bp	13	24	8/24/1999	(finback whale)
Bos taurus	NC_001567	16338 bp	13	24	8/24/1999	(cow)
Canis familiaris	NC_002008	16728 bp	13	24	8/24/1999	(dog)
Cavia porcellus	NC_000884	16801 bp	13	24	8/25/1999	(domestic guinea pig)
Ceratotherium simum	NC_001808	16832 bp	13	24	8/24/1999	(white rhinoceros)
Dasypus novemcinctus	NC_001821	17056 bp	13	24	8/24/1999	(nine-banded armadillo)
Didelphis virginiana	NC_001610	17084 bp	13	24	8/24/1999	(North American opossum)
Equus asinus	NC_001788	16670 bp	13	24	8/24/1999	(donkey)
Equus caballus	NC_001640	16660 bp	13	24	8/24/1999	(horse)
Erinaceus europaeus	NC_002080	17447 bp	13	24	8/25/1999	(western European hedgehog)
Felis catus	NC_001700	17009 bp	13	24	8/24/1999	(cat)
Gorilla gorilla	NC_001645	16364 bp	13	24	3/ 3/1996	(gorilla)
Halichoerus grypus	NC_001602	16797 bp	13	24	8/24/1999	(gray seal)
Hippopotamus amphibius	NC_000889	16407 bp	13	24	8/25/1999	(hippopotamus)
Homo sapiens	NC_001807	16569 bp	13	35	8/24/1999	(human)
Hylobates lar	NC_002082	16472 bp	13	24	8/24/1999	(common gibbon)
Lama pacos	NC_002504	16652 bp	13	24	7/26/2000	(alpaca)
Loxodonta africana	NC_000934	16866 bp	13	24	4/ 2/1998	(African elephant)
Macropus robustus	NC_001794	16896 bp	13	24	8/24/1999	(wallaroo)
Mus musculus	NC_001569	16295 bp	13	24	8/24/1999	(house mouse)
Myoxus glis	NC_001892	16602 bp	13	24	8/24/1999	(fat dormouse)
Ornithorhynchus anatinus	NC_000891	17019 bp	13	24	8/25/1999	(duckbill platypus)
Orycteropus afer	NC_002078	16816 bp	13	24	8/24/1999	(aardvark)
Oryctolagus cuniculus	NC_001913	17245 bp	13	24	8/24/1999	(rabbit)
Ovis aries	NC_001941	16616 bp	13	24	8/24/1999	(sheep)
Pan paniscus	NC_001644	16563 bp	13	24	2/ 7/1995	(pygmy chimpanzee)
Pan troglodytes	NC_001643	16554 bp	13	24	2/ 7/1995	(chimpanzee)
Papio hamadryas	NC_001992	16521 bp	13	24	8/24/1999	(baboon)
Phoca vitulina	NC_001325	16826 bp	13	24	8/24/1999	(harbor seal)
Physeter catodon	NC_002503	16428 bp	13	24	7/20/2000	(sperm whale)
Pongo pygmaeus	NC_001646	16389 bp	13	24	2/ 8/1995	(orangutan)
Pongo pygmaeus abelii	NC_002083	16499 bp	13	24	8/24/1999	(Sumatran orangutan)
Rattus norvegicus	NC_001665	16300 bp	13	24	8/24/1999	(Norway rat)
Rhinoceros unicornis	NC_001779	16829 bp	13	24	8/24/1999	(greater Indian rhinoceros)
Sciurus vulgaris	NC_002369	16507 bp	13	24	6/18/2000	(Eurasian red squirrel)
Sus scrofa	NC_000845	16613 bp	13	24	8/24/1999	(pig)
Talpa europaea	NC_002391	16884 bp	13	24	1/31/2000	(European mole)
Tupaia belangeri	NC_002521	16754 bp	13	24	9/ 1/2000	(northern tree shrew)

Analysis Coffee Break

Cytosolic help for mitochondrial defects

Order of mitochondrial genes reveals lineage

10415	n1	I	Q	M	n2	W	A	N	C	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Bos taurus
10685	n1	I	Q	M	n2	W	A	N	C	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Felis catus
10552	n1	I	Q	M	n2	W	A	N	C	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Gorilla gorilla
12188	n1	I	Q	M	n2	W	A	N	C	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Homo sapiens
10418	n1	I	Q	M	n2	W	A	N	C	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Mus musculus
15105	n1	I	Q	M	n2	W	A	N	C	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Ornithorhynchus anatinus
10470	n1	I	Q	M	n2	A	C	W	N	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Didelphis virginiana
12156	n1	I	Q	M	n2	A	C	W	N	Y	c1	S2	D	c2	K	a8	a6	c3	G	n3	R	4l	n4	H	S1	L1	n5	n6	E	cb	T	P	F	sr	V	lr	L2	Macropus robustus

Key to gene abbreviations

The table above depicts the order of mitochondrial genes for 8 of the 40 members of the class *Mammalia* with sequenced mitochondrial genomes. The gene for NADH dehydrogenase subunit 1 (n1) is used as a starting point for determining the order of genes within the mitochondrial genome (to view a key to the gene abbreviations, click on the link just below the table). Genes that code for proteins are highlighted in pink, genes that code for tRNAs are highlighted in light blue, and genes that code for ribosomal RNA are highlighted in light green. The numbers to the left of each row link to the organism's mitochondrial genome page in Entrez. Clicking on the organism name to the right of each row will display the taxonomy record for that organism.

Mitochondria are small organelles that perform functions crucial to metabolism and energy generation in eukaryotes [1]. Mitochondria maintain their own DNA which is separate from the nuclear genome. Mitochondrial DNA (mtDNA) is relatively small in size around 15-20 kb, and in multicellular organisms it is almost always in the form of circular DNA [2]. mtDNA encodes 13 enzymes involved in metabolism, 2 ribosomal RNAs, and 22 transfer RNAs that are used exclusively for the translation of mitochondrial products. Mitochondria usually use a genetic code that differs slightly from the standard genetic code.

The mitochondrial gene order is consistent among all but two of the mammals that have had their mitochondrial genomes completely sequenced. In the *Didelphis virginiana* (North American opossum) and the *Macropus robustus* (wallaroo), both marsupials, a gene rearrangement of W-A-N-C-Y to A-C-W-N-Y is present. Mitochondrial gene order rearrangements allow insight into phylogenetic relationships between organisms, and in this case provides evidence for how the three classes of mammels Eutheria (placentals), Monotremata (egg-laying mammals), and Marsupialia (marsupials) diverged [3, 4]. First, the Eutheria diverged from the Monotremata and Marsupialia 130 million years ago; then, 15 million years later, the Marsupialia and Monotremata diverged. This explains how the wallaroo and opossum, even though they are continents apart in Australia and North America, share the same gene order, whereas the duck bill platypus (a member of Monotremata and resident of Australia), has the same order as the Eutheria [3, 4].

[1] Brand, MD (1997) Regulation analysis of energy metabolism. *J Exp Biol* 200, 193-202.

[2] Boore, JL (1999) Animal mitochondrial genomes. *Nucleic Acids Res* 27, 1767-80.

[3] Janke A, *et al.* (1997) The complete mitochondrial genome of the wallaroo (Macropus robustus) and the phylogenetic relationship among Monotremata, Marsupialia, and Eutheria. *Proc Natl Acad Sci USA* 94(4), 1276-81.

[4] Janke A, *et al.* (1996) The mitochondrial genome of a monotreme-the platypus (Ornithorhynchus anatinus). *J Mol Evol* 42, 153-9.